INTEGRATED RESEARCH PROGRAM IN SPACE NUTRITION

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Co-Principal Investigators:

Doris Howes Calloway
Professor of Nutrition

Sheldon Margen
Professor of Human Nutrition

Rosemarie Ostwald
Associate Professor of Nutrition

Department of Nutritional Sciences

University of California, Berkeley

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Abstract: Nutrition and Breeding Behavior of the Pocket Mouse

1. Baseline data for the growth of P. pe. fed a diet of mixed seeds were established. This was intended as a preliminary for the determination of the adequacy for growth of our semi-synthetic diet.

2. The study of the effects of photoperiod on estrus behavior of P. pe. was terminated. The data of this 3-year study have been compiled and a manuscript for publication is in preparation.

3. A study of the intestinal absorption and metabolism of calcium, magnesium, and phosphorus by P. pe. has been undertaken.

NOTE: Because of financial restraints during this project period a technician was available for five months only.

Report - Work Completed

Nutrition and Breeding Behavior of the Pocket Mouse

1. Adequacy of the semi-synthetic diet for growth of P. penicillatus

We have previously shown that the semi-synthetic diet developed by us is adequate for weight maintenance and general health of adult pocket mice (Spiller, Gene A. and Rosemarie Ostwald, J. Nutr. 94:297, 1968). In order to test the adequacy of this diet for growth it was necessary to establish as a baseline growth of these animals on their usual diet.

Ten litters of P. pe. (19 males, 13 females) born during summer and fall 1969 were used in this study. The dams and pups were fed the mixed seed diet routinely used by us (equal parts of sunflower seeds, millet, canary seeds and oats) and were supplemented with fresh carrots and lettuce 5 times/week. By previous experience, these supplements have been found necessary for reasonable survival of the young. In the absence of carrots,

which we believe to serve primarily as source of water, there is little growth and a high mortality, probably due to failure of lactation. In the absence of lettuce there is a high rate of mortality at about 20-25 days of age preceeded by characteristic overactivity and convulsions. We believe that this may be caused by a magnesium deficiency, alleviated by the lettuce.

The pups were marked (1% gentian violet) and weighed 3 times/week, starting at about 14 days of age. We did not attempt earlier weighing for fear of disturbing the dams unduly and so inhibit lactation. The pups were separated into individual cages when they reached 10-11 g. Earlier separation led to lack of growth and high mortality.

Figures 1 and 2 show the growth curves. There was rapid growth to about 20 days of age (0.6 and 0.5 g/day for males and females respectively). After this they grew at a slower rate to 45-55 days (0.16 and 0.13 g/day for males and females respectively).

There were 14 deaths during the 80 days of the experiment. Table 1 shows the age and sex distribution of these losses. Males seemed to be less sturdy than females (10 males vs. 4 females). The cause of these deaths are unknown. Visual inspection at autopsy did not reveal any obvious abnormalities. Twelve of the 14 deaths occurred in only 5 of the 10 litters. In most cases the animals lost weight during the week preceding death and just did not seem to thrive.

2. Mineral metabolism of P. Pe.

We have previously shown (Spiller, Gene A. and Rosemarie Ostwald, J. Nutr. 94: 297, 1968) that a diet containing calcium, magnesium, and phosphorus in the inorganic forms usually employed is tolerable for pocket mice only if supplement with carrots as source of water. If, however, calcium- and magnesium-glycero phosphate are used as source of minerals the diet is adequate without such supplementation. In order to understand

the reasons for this observation, a study was undertaken to investigate the intestinal absorbtion of the macro minerals in these animals and their subsequent metabolic fate. The plan is to measure the intestinal absorbtion, body retention and excretion of calcium, magnesium and phosphorus using radioactive isotopes in P. Pe. fed four different diets:

1) Mixed seeds, on which they can be maintained indefinetly.

2) The lethal diet containing inorganic salts.

3) The same diet with a supplementation of carrots.

4) Our semi-synthetic diet containing calcium- and magnesium-glycero phosphates.

As necessary preliminaries we have studied:

a) Methods for the collection of feces and urine.

b) The amounts of calcium, magnesium and phosphorus excreted in feces and urine.

c) The administration of the tracers.

d) Methods to determine the amounts of radioactivity excreted and retained.

Results

Fecal pellets can be collected from mice kept in glass metabolic cages for 24 hours without food. Urine can be obtained by washing the cages. The amounts of calcium and magnesium in the excreta has been determined by atomic absorbtion measurement of dried and wet-ashed feces and in the urine samples in 1% $LaCl_2$ (Table 2).

In order to administer the radioactive tracer a small pellet of the appropriate diet was treated with an aequous solution of the tracer, then dried, counted and placed on the back of the tongue of the mouse who swallowed it by reflex action.

We have chosen to study initially Ca^{47} because of its desirable half-life and energy characterisitics. Figure 3 shows that body retention and decay

of Ca^{47} can be measured by whole body counting in the well of a Packard auto-gamma spectrometer, series 410A.

We have also studied the possibility of measuring P^{32} by its bremstrahlung radiation characteristics. Figure 4 shows that this is indeed possible in spite of the fact that the peak of the energy distribution occurs at the lower end of the scale where the noise level of the machine is high. With a wide mode of analysis (200-1000 or 100-1000 Kev.) the results may be even better. This will make it possible to use P^{32}, a β-emitter, in the same fashion as Ca^{47} for the study of phosphate metabolism.

Table 1

Mortality of Young P. Pe.

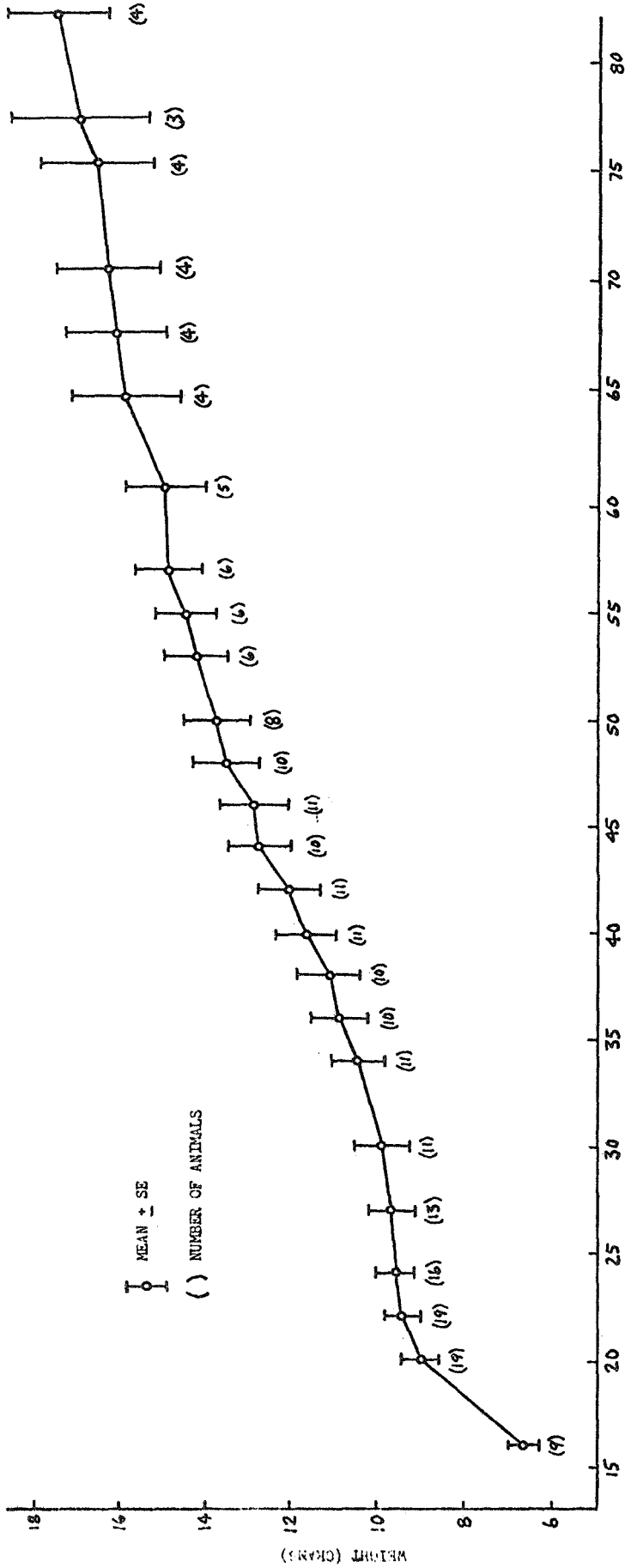
Age (days)	14-25	26-35	36-45	46-65	56-80	Total
Male (19)	0	5	0	5	0	10
Female (12)	0	0	1	2	1	4
Male + Female	-	5	1	7	1	14

Table 2

Excretion of Calcium and Magnesium in P. Pe

	Animal	Calcium mg/24 hrs.	Magnesium mg/24 hrs.	Calcium mg/24 hrs/mg dry wt.	Magnesium mg/24 hrs/mg dry wt.
Urine	1	293	94	-	-
	2	506	97	-	-
	3	501	-	-	-
	4	453	186	-	-
Feces	2	687	1132	20	33
	3	407	1232	9	27
	4	455	832	16	29

FIGURE 1. GROWTH OF P. PE. (MALES)



Note: The variable numbers of animals for each point are due to deletion of data when an animal suffered a temporary weight loss or when a litter was removed from the experiment and due to losses by death.

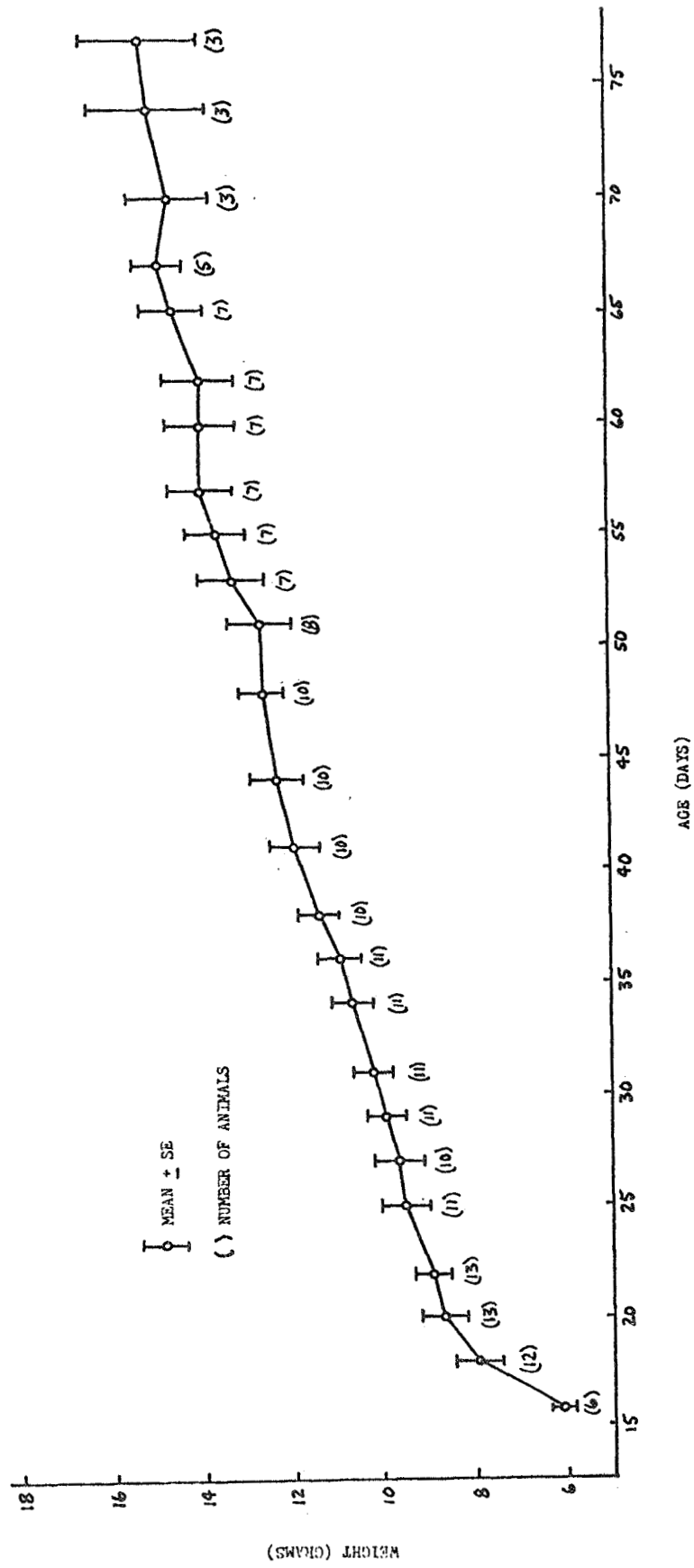
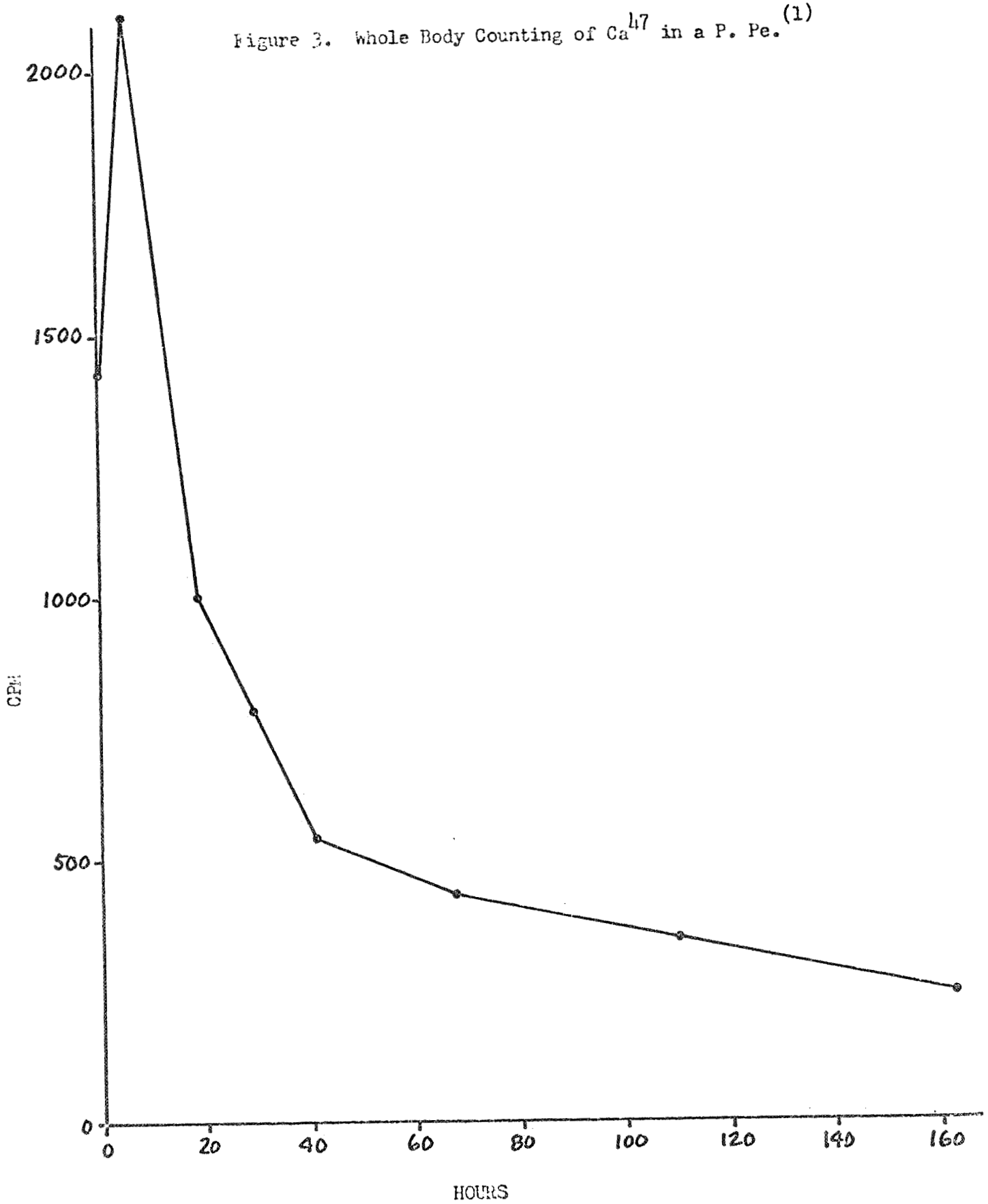


FIGURE 2. GROWTH OF *P. PL.* (FEMALES)

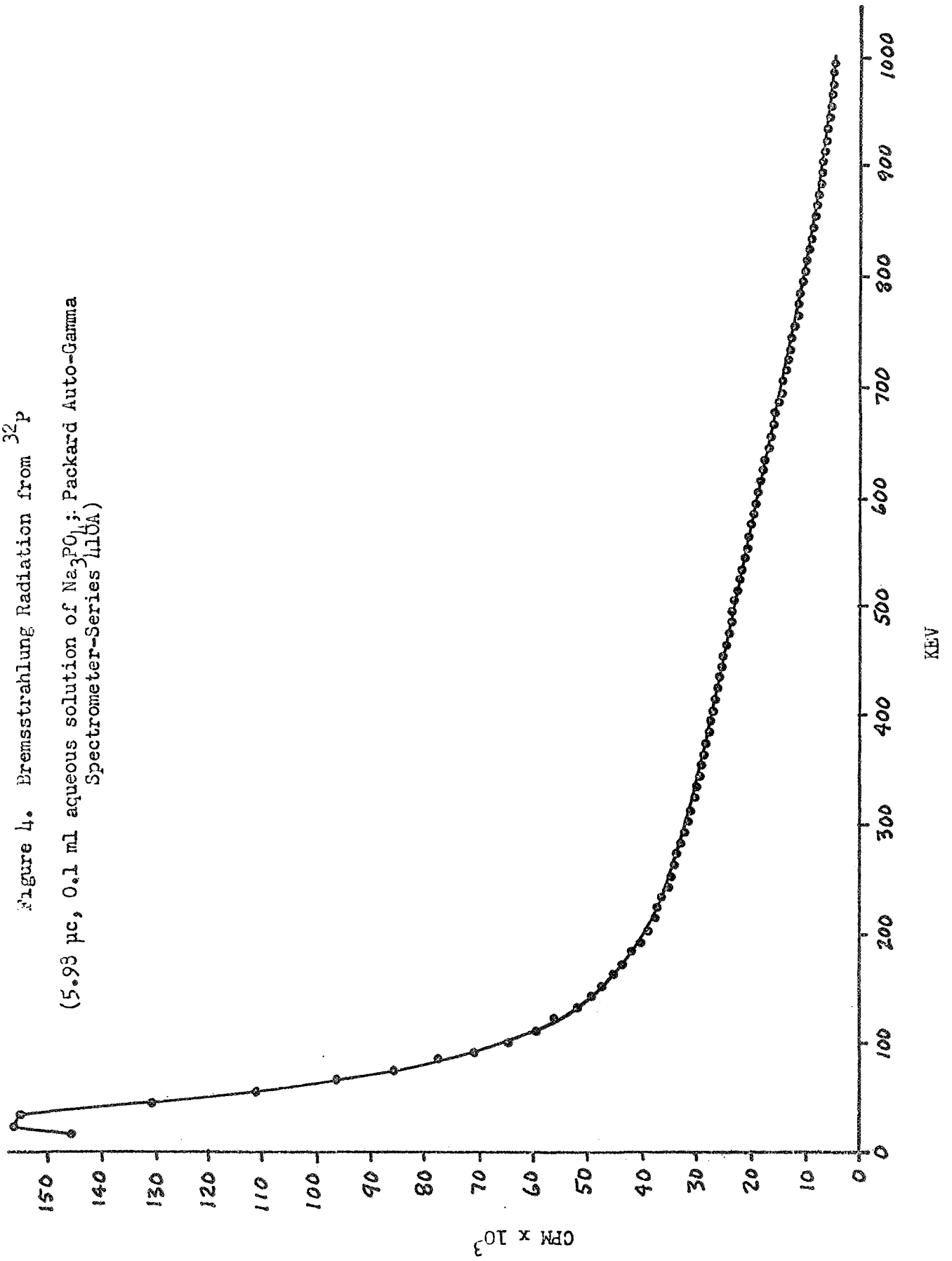
Figure 3. Whole Body Counting of Ca^{47} in a P. Pe.(1)



(1) A pellet of gly-7 diet containing Ca^{47} and counting 5000 cpm (in the same well in which the mouse was counted) was swallowed by the mouse at 0 hours.

Figure 4. Bremsstrahlung Radiation from ^{32}P

(5.93 μc, 0.1 ml aqueous solution of Na_3PO_4; Packard Auto-Gamma Spectrometer-Series 410A)



WORK IN PROGRESS

1. Efficient Substrates for Energy.

In an attempt to determine the minimal amounts of carbohydrate, fat and protein necessary to prevent tissue catabolism, studies were carried out on six obese subjects. Obese subjects were studied because feeding such subjects the major foodstuffs at low caloric intake is probably the best model to answer questions of ideal energy substrates. The obese subjects have a large supply of energy source--their own adipose tissue. They are also able to adapt to fatty acids as the major energy source. Experimental evidence has suggested that the obese fasting individual is able to most efficiently burn fat and conserve protein. We also attempted to asnwer the question whether utilization of adipose tissue for energy is always accompanied by a mandatory breakdown of cellular protein. The experimental plan diets are outlined below (Fig. 5).

The results of the experiment are still being analyzed. However, the following results can be commented upon:

1) All subjects lost weight at about the same rate.
2) The weight loss appeared to be related to caloric deficit and not to the substrate for energy being fed.
3) With weight loss there was a gradual fall in metabolic rate. This decrease was due to (a) decrease in active metabolic tissue and (b) apparently an alteration in efficiency of utilization of substrate. The latter statement is a difficult to substantiate and a more careful analysis of the data is underway before we can be certain of this statement.

Analysis of the body composition changes is not yet completed. Correlations between various techniques of measurement appear to be low.

Figure 5

Final Diet Plan, Experiment #16 (April - June, 1969)

Metabolic Period	I	II	III	IV	V	VI
No. Days/Period	12	12	9	12	12	7
Dates	4/21-5/2	5/3-5/14	5/15-5/23	5/24-6/4	6/5-6/16	6/17-6/23
Subjects	Diets					
Mabel A.	#1	#2	#4	#3	#5	1000-cal.food[1]
Ruth M.	#1	#2	#3	#4[2]	--	---
Astrid B.	#1	#4	#3	#2	#5	1000-cal.food
Meta D.	#1	#3	#4[3]	#2[4]	#5	1000-cal.food
Malinda L.	#1	#4	#2	#3	#5	1000-cal.food
Mary F.	#1	#3	#2[3]	#4[4]	#5	1000-cal.food

	Approx. gN/day
Diet #1 = 2000 Kcal, egg albumin + fat equal to Diet #3	12.4
Diet #2 = 1000 Kcal, egg albumin and only CHO	12.4
Diet #3 = 1000 Kcal, egg albumin and only fat	12.3
Diet #4 = 1000 Kcal, all protein (egg albumin, promine + casal)	38.8
Diet #5 = 377 Kcal, egg albumin	12.3

COMPOSITION OF DIETS AS SHOWN IN FIGURE 6.

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1) 1000 calorie food diet - balanced meals of low-calorie food items to equal about 1000 calories, 100 gm protein/day, and minimal fat.
2) left 5/24.
3) 5/15-5/26.
4) 5/27-6/4.

Figure 6

Composition of Diets.

Diet #2: 1013 Kcal.
12 gm N as egg albumin.
Rest of Kcal as CHO.
650 Kcal of the 1013 as CHO Kcal = 64.2%.
Also 28 Kcal as fat.
Also 328 Kcal as protein.

Diet #3: 1010 Kcal.
12 gm N as egg albumin.
Rest of Kcal as fat (50% butterfat, 50% safflower oil).
662 Kcal of the 1010 as fat Kcal = 65.5%.
Also 19 Kcal as CHO Kcal.
Also 327 Kcal as protein.

Diet #4: 1015 Kcal - "all" protein.
946 Kcal of 1015 as protein Kcal
(comes from 12.0 gm N egg albumin
13.9 gm N promine D
12.62 gm N casal).
Also 3 gm safflower oil = 27 fat Kcal.
+ 15 Kcal from fat in 3 protein sources.
Also 28 Kcal as CHO Kcal in protein sources.

2. Creatine and Creatinine Pool Size and Turnover Rate.

The experiment relating to creatine and creatinine pool size and turnover rate is in progress. The human feeding portion has been completed and analyses of heavy isotope labelled compounds are under way.

3. Utilization of Ketone Bodies in Brain During Starvation.

The brain was formerly considered to utilize glucose only as energy fuel. However, recent evidence, especially the study of prolonged starvation treatment of obesity patients, have shown that ketone bodies were the major energy source after a long starvation. Our preliminary studies showed that the brain could utilize acetoacetate or β-hydroxybutyrate in vitro and that the amount of acetyl Co-A formation was increased in the brain preparation of starving animals. We measured the amount of various metabolites of the glycolytic pathway in various conditions in order to determine the enzymes responsible for regulation. Enzyme levels were also measured in either isolated subcellular particles or histochemical preparation. No definite and conclusive mechanism could be obtained at this moment because of inconsistent results and the interference of glucose and glycogen in the preparation. The current attempts to inhibit the utilization of glucose for this study have not been very successful.

Further studies to eliminate the effect of glucose and glycogen, using eviscerated animals and cytochemical studies for various different compartments of the brain should be performed in order to elucidate the mechanism of utilization of ketone bodies in the brain.

FUTURE PLANS

Work on isolation of the radiation protective factor from alfalfa has been temporarily suspended pending search for an improved isolation procedure.

If additional funds are made available, work will be continued on efficient use of substrates for energy.

Current funds will be sufficient to complete the study of creatine and creatinine pool size and turnover rate.